

Intracellular nucleic acid sensing

Brian Ferguson

Part II Immunology

22/10/2103

Pattern recognition receptor signalling

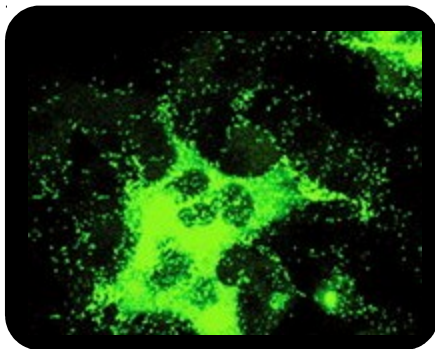


1) Direct detection of pathogens:

- Molecular discrimination between self and non-self - PAMPs
- Sounds the infection 'alarm'

2) Detection of injury or tissue damage:

- Inflammatory response to DAMPs
- Restores homeostasis



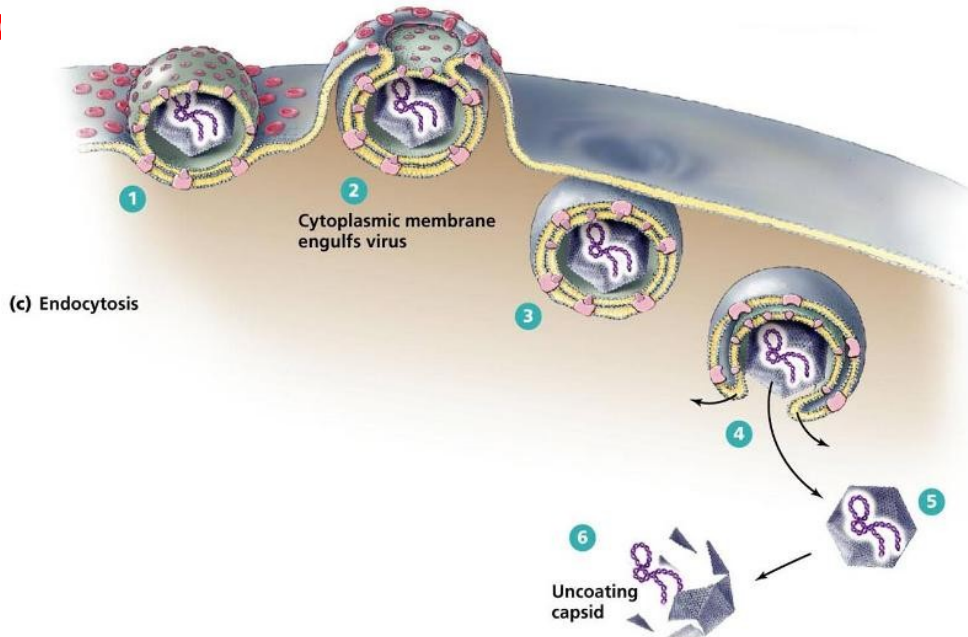
Require **specific sensors** but induce the **same mediators** - secretion of interferons, cytokines, chemokines , AMPs etc.

Intracellular nucleic acid sensing of infection

The production of type 1 interferons in response to virus infection *in vivo* is critically dependent on **nucleic acid** sensing in non-hematopoietic cells

Even though there are many PAMPS in a single virus – detection of the genome is crucial to sensing and responding to the presence

C

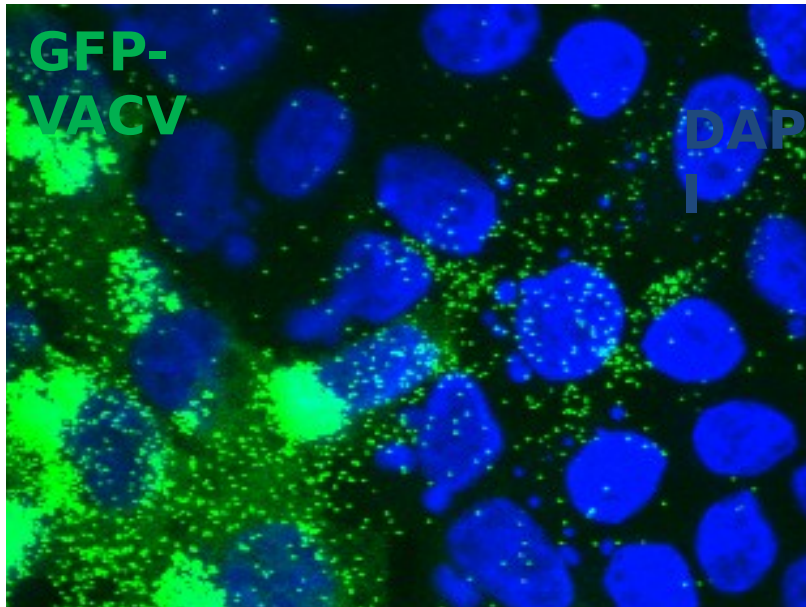


Genome must be exposed for replication to occur

Although this can happen in many different ways depending on the virus

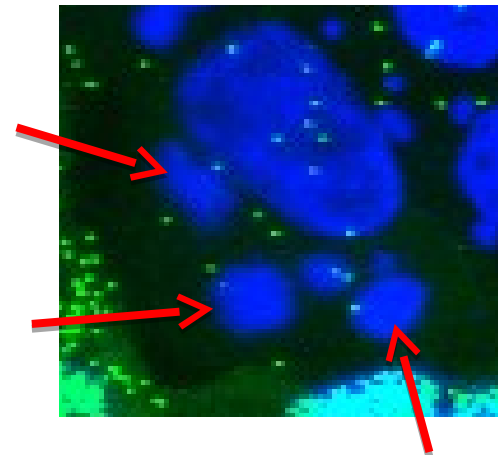
Innate immune response to viral DNA

viruses 2 types: DNA (or RNA) genomes – Vaccinia virus is a DNA virus



Vaccinia DNA
'factories'

**VACV replicates its
200kb DNA
genome in the
cytoplasm**



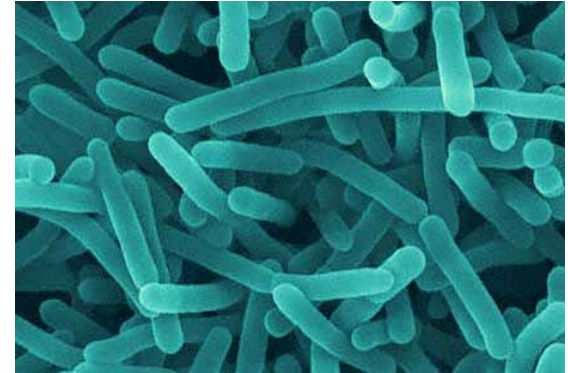
Viral DNA

Intracellular nucleic acid sensing

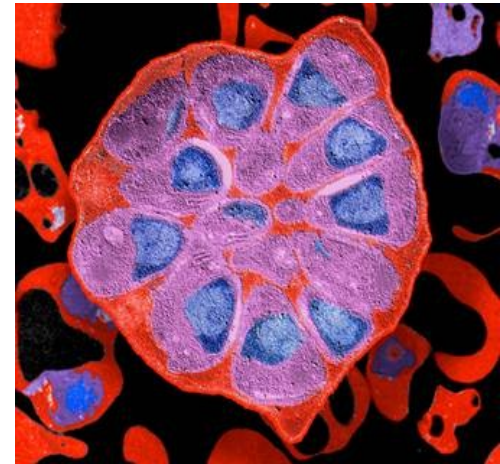
Not just viruses

Bacteria – some are intracellular
–

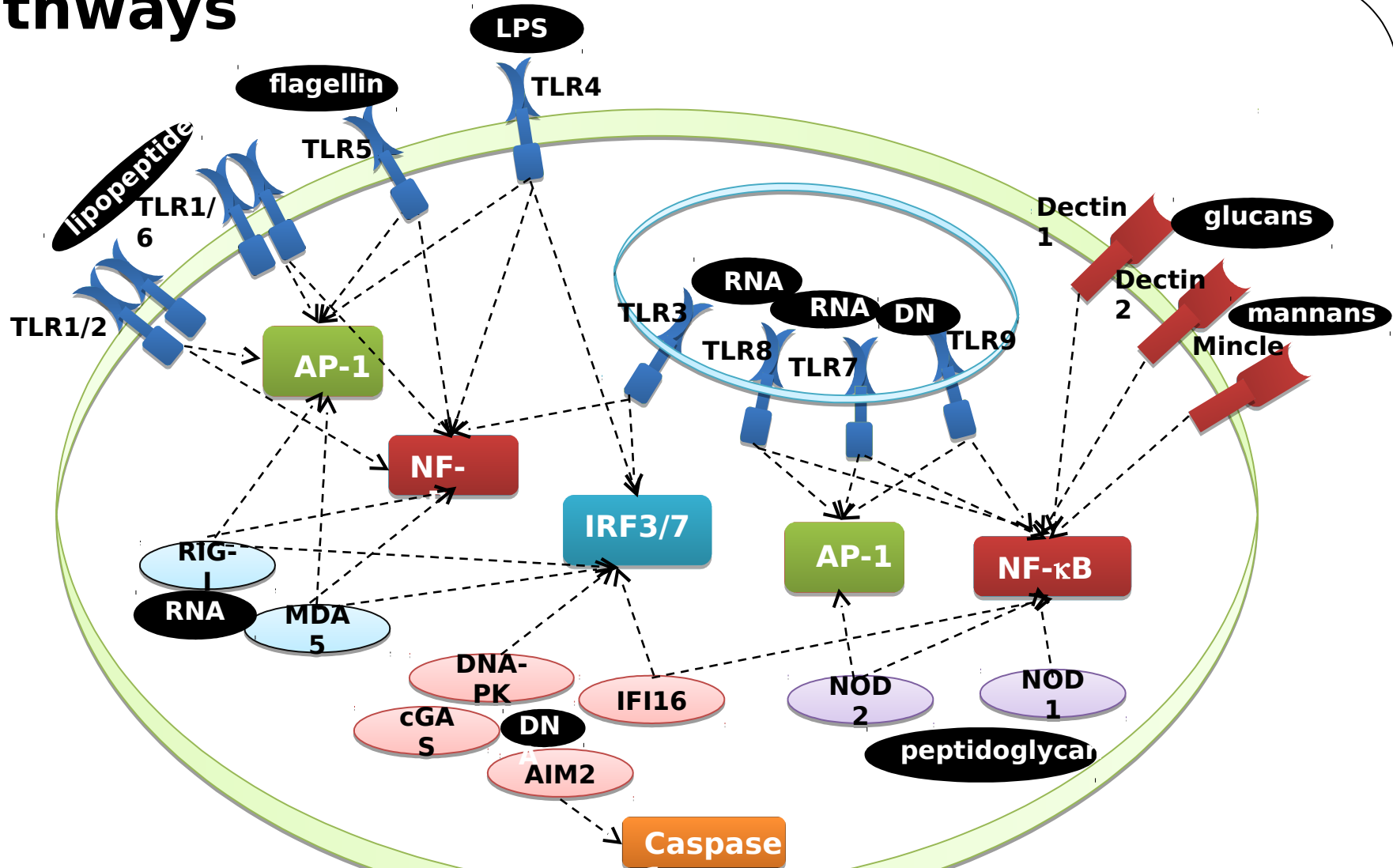
Listeria inject its DNA and RNA
into cytoplasm following
infection



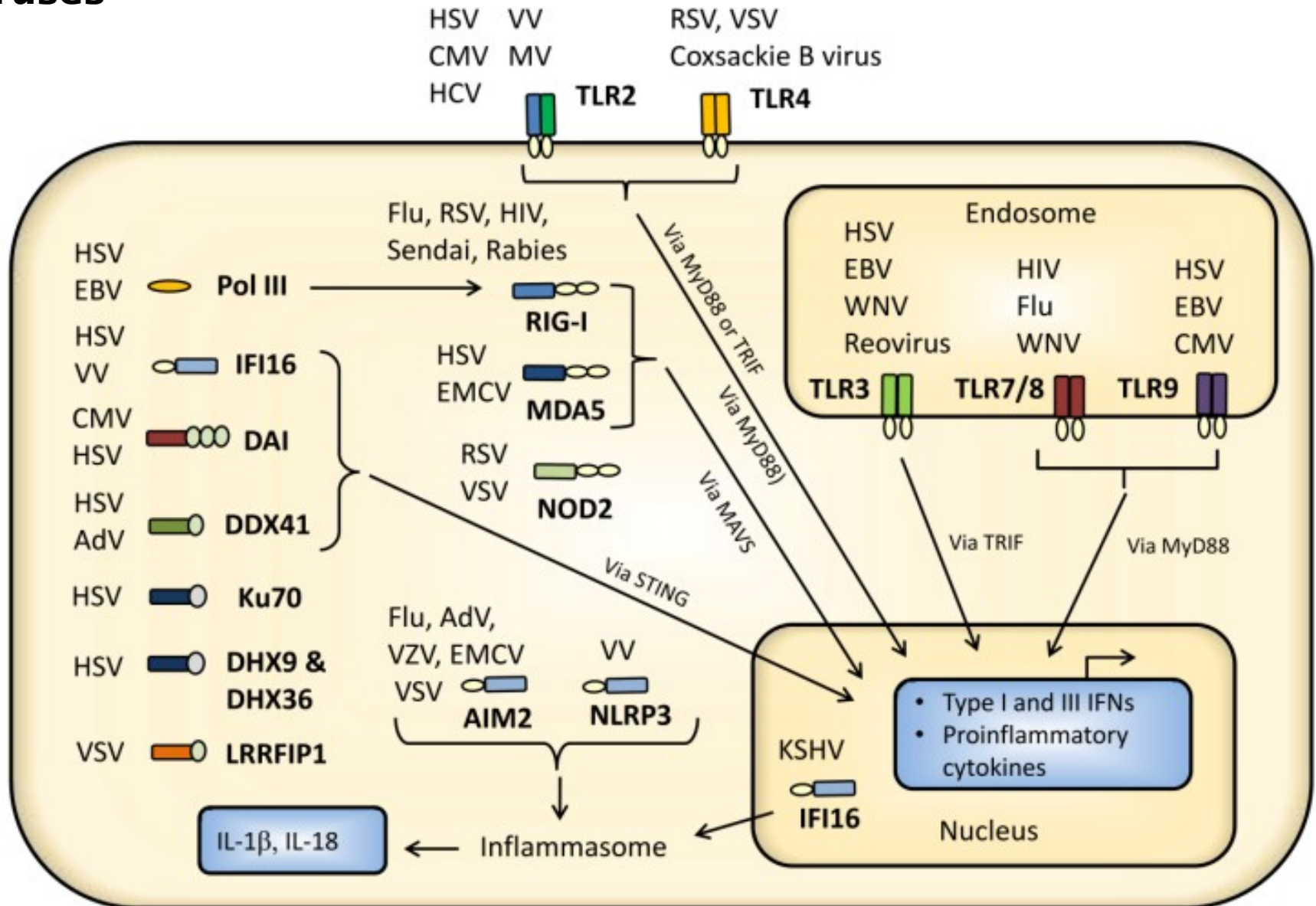
Protazoa – eg plasmodium
falciparum
DNA genome sensed by innate
immune system



Pattern recognition molecular pathways



PRR sensing of viruses



Viral RNA sensing

RIG-I

(-)ssRNA

Paramyxoviridae: (-) ssRNA, NS

Sendai virus (SeV) (Andrejeva, 2004; Kato, 2005; Yoneyama, 2005; Strahle, 2006; Baum, 2010)
Newcastle disease Virus (NDV) (Kato, 2005)
Respiratory syncytial virus (RSV) (Loo, 2008)
Measles virus (MV) (Plumet, 2007)
Nipah virus (NiV) (Habjan, 2008)
Parainfluenzavirus 5 (PIV5) (Childs 2007; Childs 2012; Motz, 2013)

Filoviridae: (-) ssRNA, NS

Ebola virus (Cardenas, 2006; Habjan, 2008)

Rhabdoviridae: (-)ssRNA, NS

Rabies virus (RV) (Homung, 2006)
Vesicular stomatitis virus (VSV) (Kato, 2005; Yoneyama, 2005)

Defective interfering RNA (copy back)



L-tr-RNA readthrough/trRNA hybrids?



Orthomyxoviridae: (-)ssRNA, S

Influenza A virus (Kato, 2006; Pichlmair, 2006; Rehwinkel, 2010; Weber, 2013)
Influenza B virus (Loo, 2008)

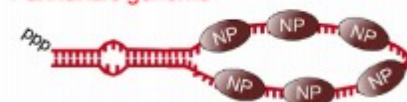
Bunyaviridae: (-) ssRNA, S

Rift Valley fever virus (RVFV) (Habjan, 2008; Weber 2013);
LaCrosse virus (LACV) (Weber 2013)

Arenaviridae: (-) ssRNA, S

Lassa virus (LASV) (Habjan, 2008)

Panhandle genome



(+)ssRNA

Flaviviridae: (+)ssRNA

Hepatitis C virus (HCV) (Saito, 2007)
Japanese encephalitis virus (Kato, 2006)
Dengue virus (Loo, 2008)
West Nile virus (Loo, 2008)

Coronaviridae: (+)ssRNA

Murine Hepatitis Virus (Li, 2010)*

dsRNA replicative intermediate?



dsRNA

Reoviridae: dsRNA, S

Orthoreovirus (Loo, 2008)

dsRNA genome?



dsRNA of DNA Viruses:

Herpes simplex virus (HSV1)

(Weber, 2006; Melchjorsen, 2010; Xing, 2012)

Adenovirus

(Pichlmair, 2009; Minamitani, 2011)

Epstein-Barr virus

(Samanta, 2008; Ablasser, 2009)

MDA5

(+)ssRNA

Picornaviridae: (+)ssRNA

Encephalomyocarditis Virus (EMCV) *** (Gitlin, 2006; Kato, 2006; Feng 2012)
Theiler's virus (Kato, 2006)
Enteroviruses (Feng, 2012; Triantafyllou, 2012)
Saffold virus 3 (Feng, 2012)
human parechovirus 1 (Feng, 2012)
equine rhinitis A virus (Feng, 2012)

Calciviridae: (+)ssRNA

Norovirus *** (McCartney, 2008)

dsRNA replicative intermediate



Coronaviridae: (+)ssRNA

Murine Hepatitis Virus (Roth-Cross, 2008)*

-RLR escape-

Bunyaviridae: (-) ssRNA, S

Hantaan virus (HTNV)** (Habjan, 2008)
Crimean-Congo hemorrhagic fever virus (CCHFV; Nairovirus)* (Habjan, 2008)

Bornaviridae: (-) ssRNA, NS

Borna disease virus (BDV)** (Habjan, 2008)

Coronaviridae: (+)ssRNA

Murine Hepatitis Virus (Zhou, 2007)**













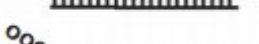





Arenaviridae: (-) ssRNA, S

Tacaribe arenavirus (TCRV) (Marq, 2010) ****

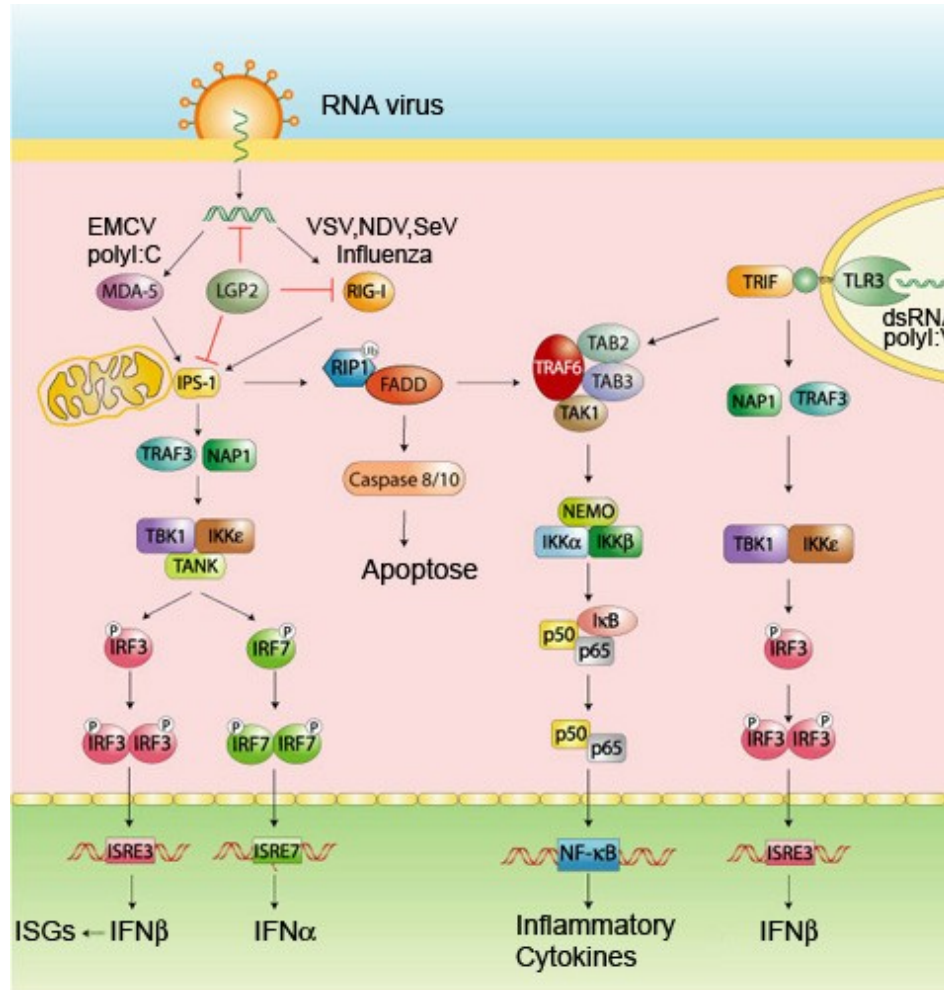
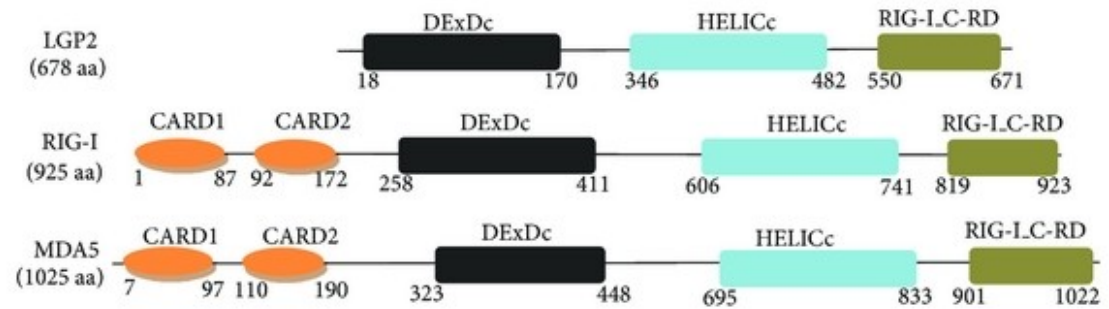
RIG-I RNA ligands

What is the physiological ligand during infection

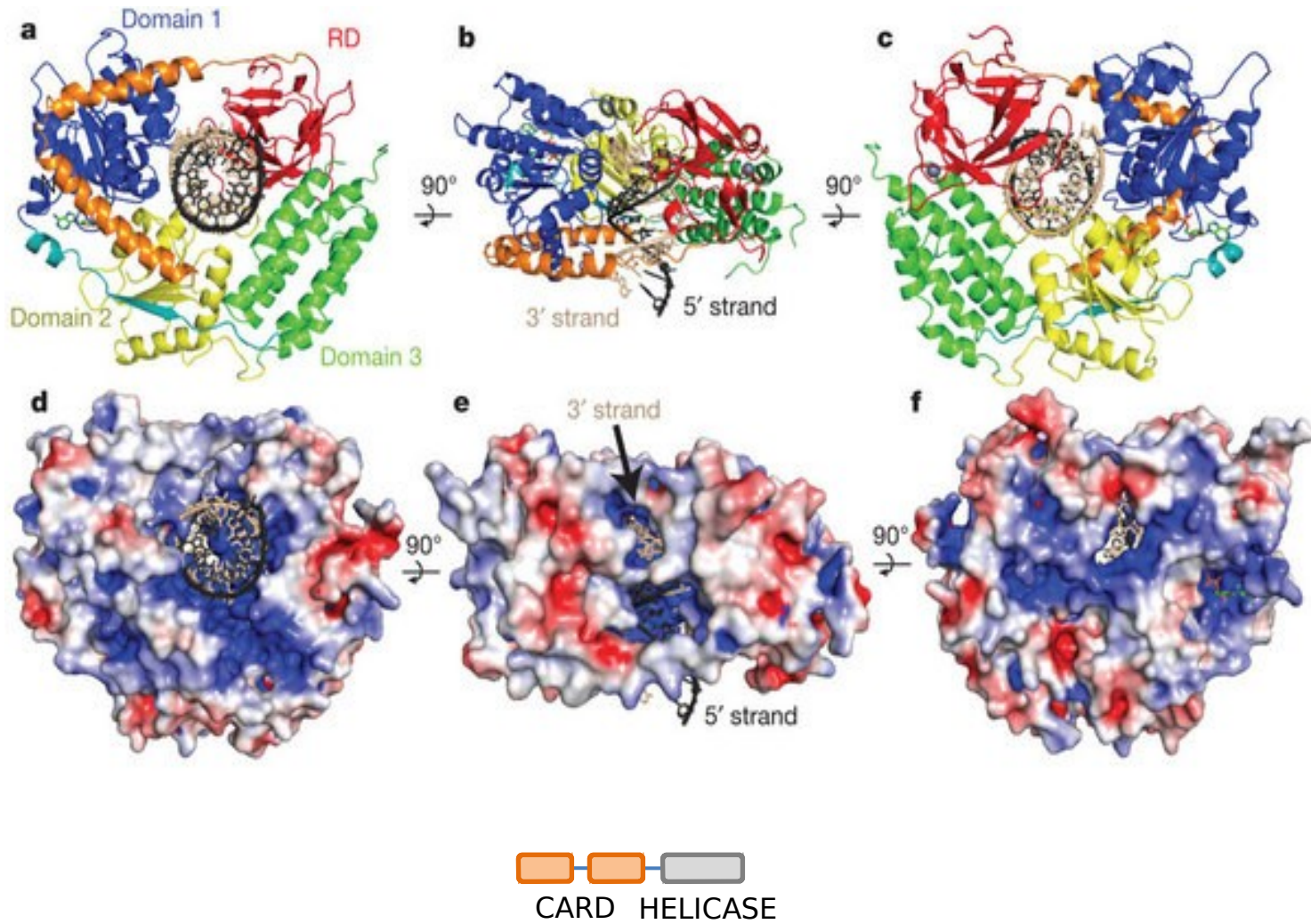
Still an open question

Marques et al., 2006 dsRNA ≥ 23 bp, 5'overhangs (both ends) strongly reduce, 3'overhangs (both ends) abolish activity p56 (IFIT1) 48h or 72h after stimulation in human cell lines (T98G, IFN- α treated HT1080)	    	++++ +++ ++++ + no
Takahasi et al., 2008 5'p-dsRNA (analysis of ≥ 25 bp) 3'overhangs (at 5'p) abolish activity (no 5' overhang tested) 3'p-dsRNA, 3'overhangs (at 3'p) accepted, (no 5' overhang or blunt end tested) IRF-3 dimerization in MEFs, IFN- β reporter assay in murine cell line (L929), binding to RIG-I (gel shift: no impact of 5'p on RIG-I binding), ATPase assay with RIG-I protein	   	++++ ++++ no ++++
Schlee et al., 2009 5'ppp-dsRNA ≥ 19 bp, pppN= A, G, U, C, bulge loops accepted. 3'overhangs (at 5'ppp) strongly reduce, 5'overhangs (at 5'ppp) abolish activity. IFN- α in human monocytes, IFN- β in MEFs, quantitative binding assay (AlphaScreen), ATPase assay with RIG-I protein	    	++++ ++++ ++++ + no
Schmidt et al., 2009 5'ppp-dsRNA ≥ 10 bp 5'overhangs =1 (at 5'ppp) accepted 5'overhangs >1 (at 5'ppp) abolish activity. (interpreted as acceptance of 5'ppp-overhangs) Induction of IFN- α in human monocytes, quantitative binding assay (fluorescence anisotropy), ATPase assay with RIG-I protein	   	(+++) (+++) (+++) no

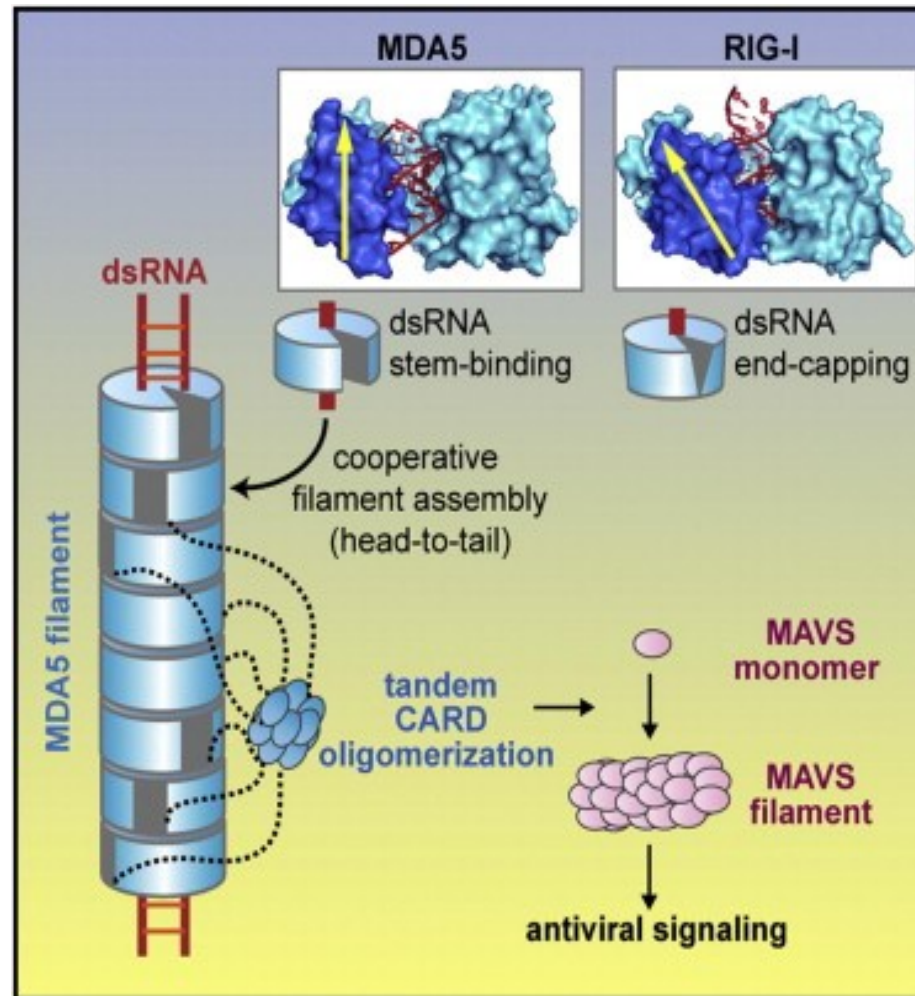
RLR signalling



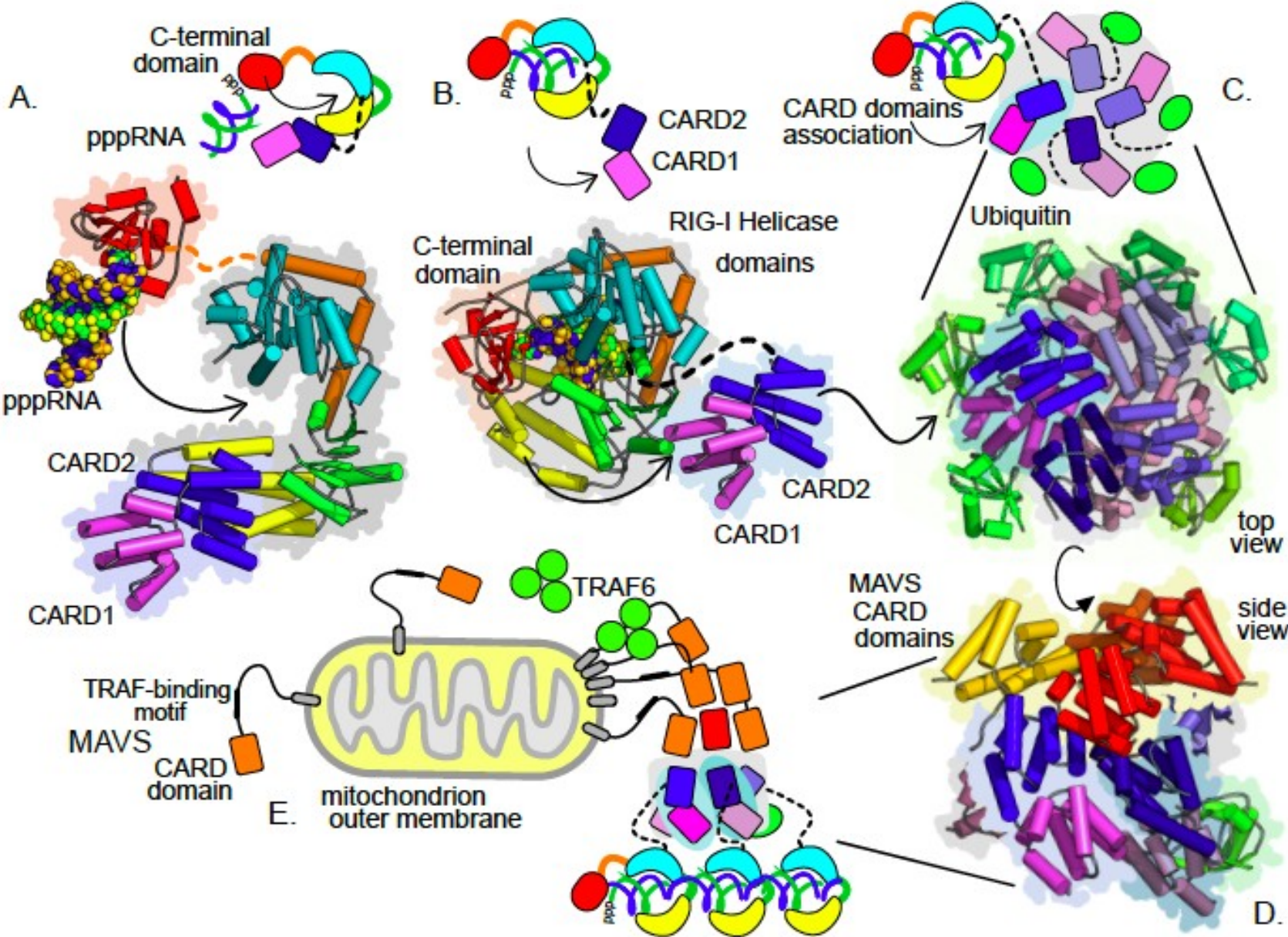
RIG-I recognition of dsRNA



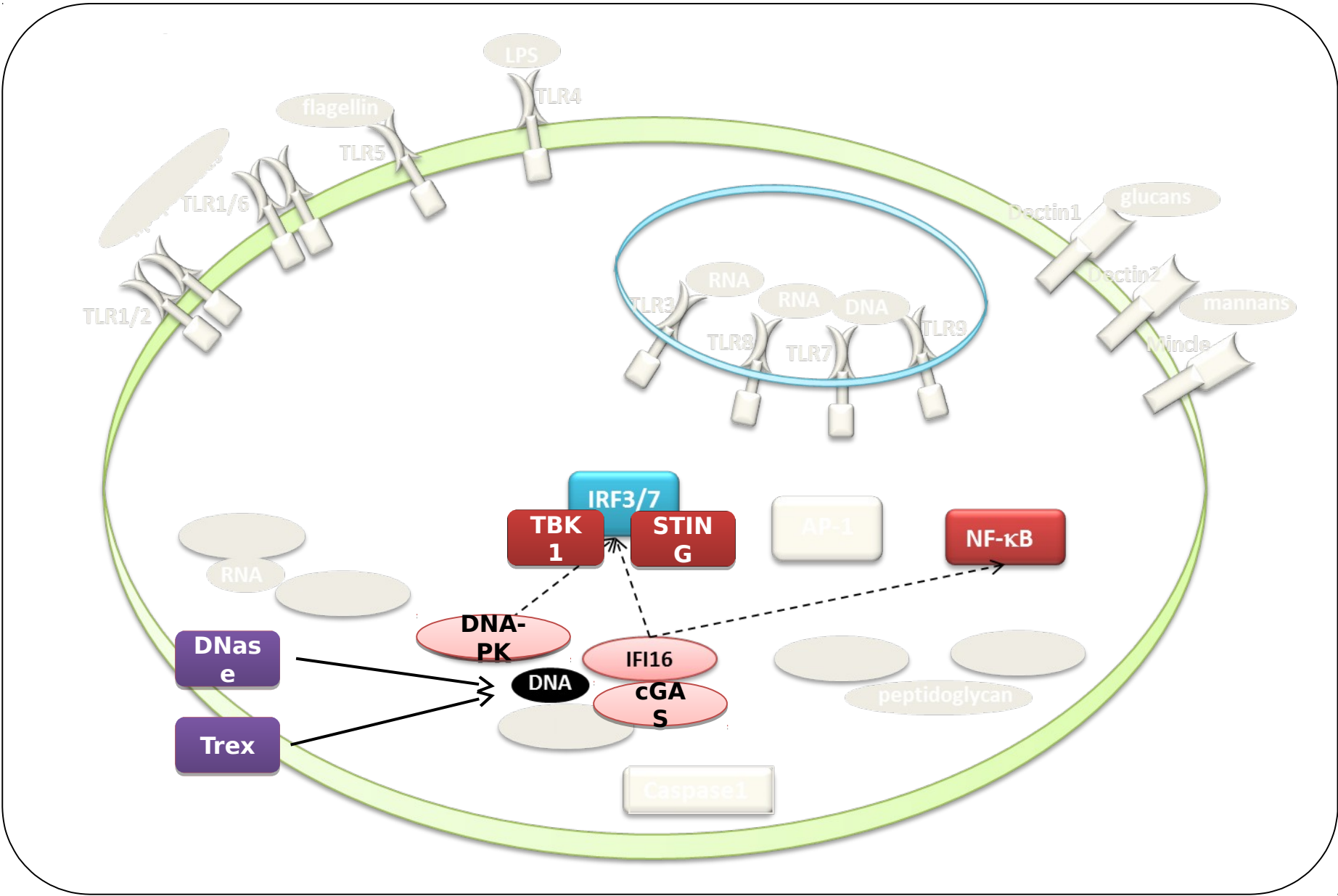
MDA-5 structure



RLR signalling activation mechanisms

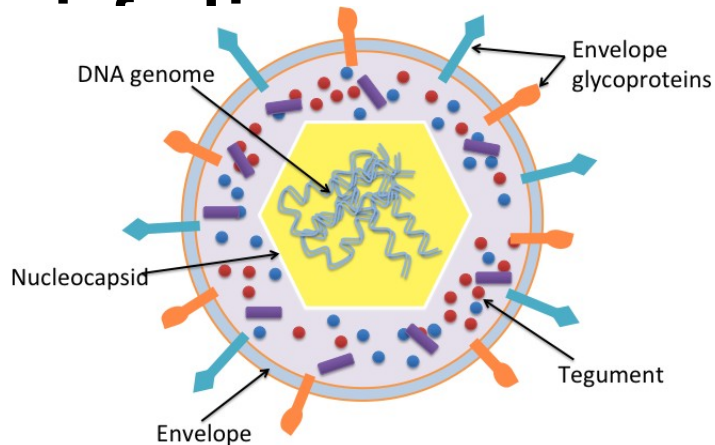


Pattern recognition - DNA

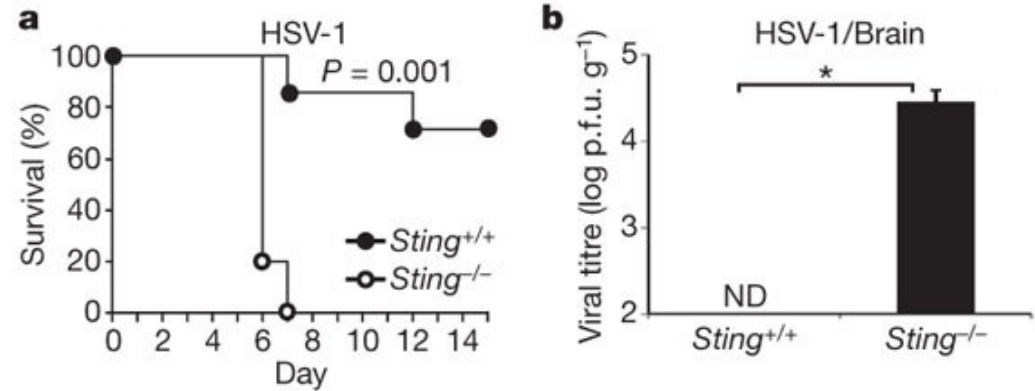


1) Immune response to pathogens

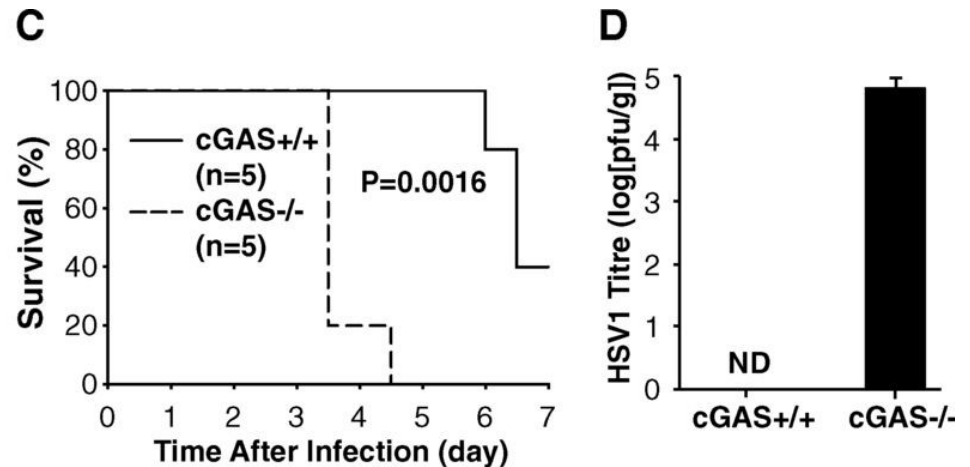
Intracellular innate immune DNA sensing is required for protection against DNA virus



Herpes Simplex virus 1



Nature. 2009 Oct 8;461(7265):788-92.

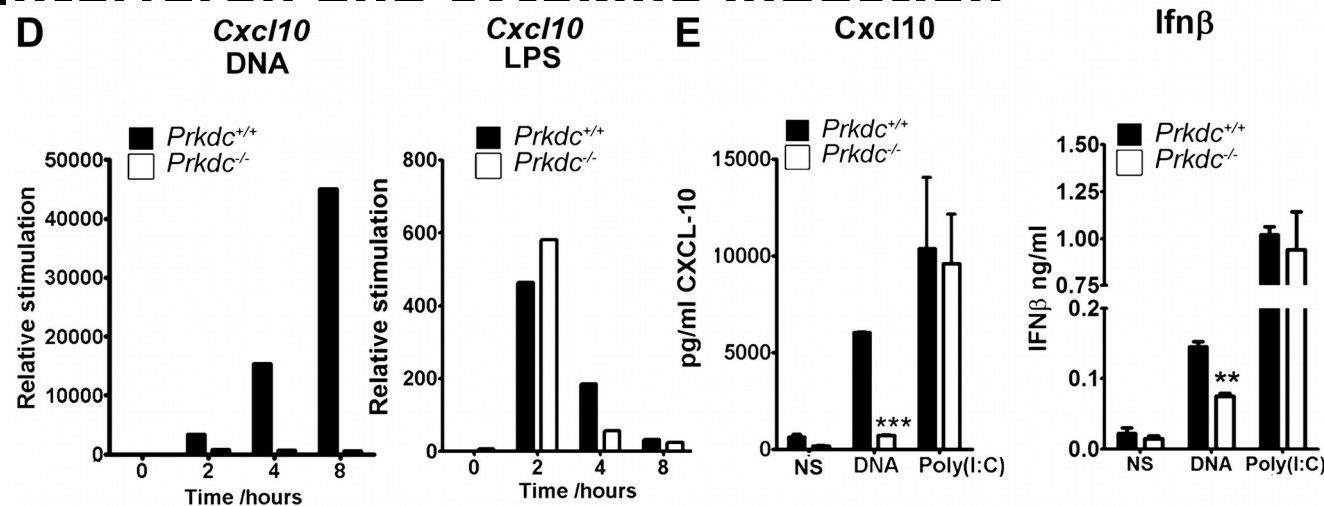


Science 20 September 2013: Vol. 341 no. 6152 pp. 1390-1394

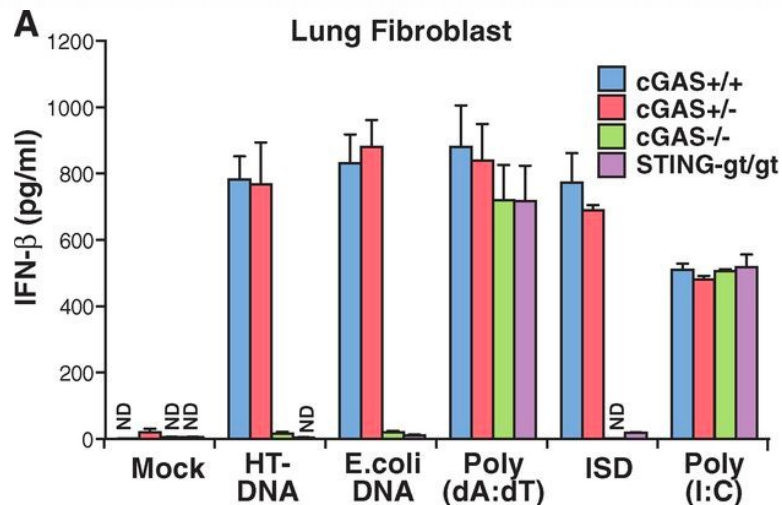
STING $-/-$, cGAS $-/-$ and IRF3 $-/-$ mice are highly susceptible to infection with HSV-1

1) Immune response to pathogens

Intracellular innate immune DNA sensing is required for DNA and DNA-virus induced Interferon and cytokine induction



Elife December 2012; 00047



Cells lacking DNA-PKcs, cGAS, IRF3 or TBK1 do not make cytokines in response to intracellular DNA stimulation or DNA virus infection

Enzymatic regulation of DNA levels

Appropriate inflammatory response results in the ability to fight infection without inappropriate responses to self

Hence there is exquisite regulation of these

processes Nucleases regulate levels of circulating and cellular nucleic acids

- DNase I, DNase II and Trex are all responsible for breakdown of DNA
- This effectively stops the innate immune response from starting until a threshold point is reached

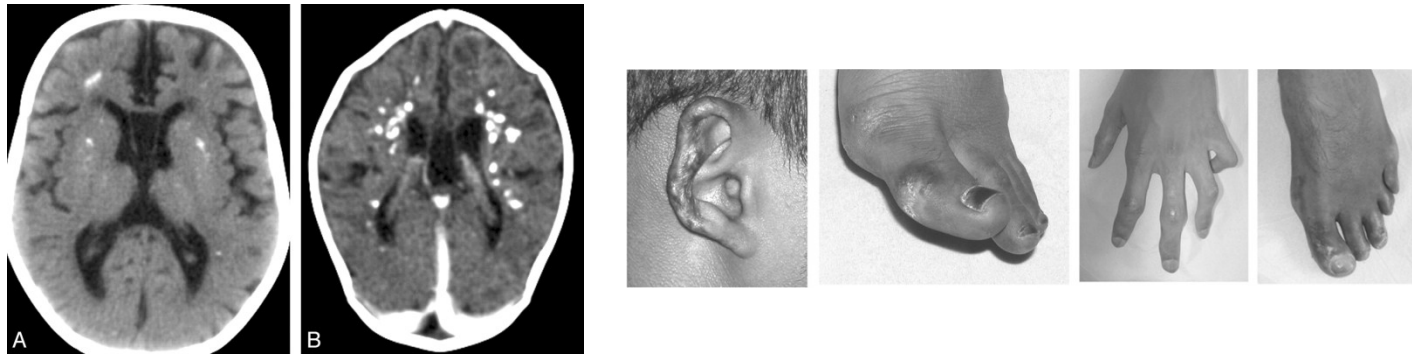
Without DNase, self DNA triggers inflammation
Dnase II knockout mice get spontaneous TNF driven 'arthritis'

DNase II^{-/-} Sting^{-/-} do not exhibit signs of arthritis



Autoimmunity from Trex mutation

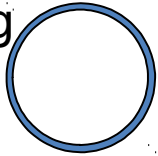
- **Aicardi-Goutières syndrome** - autoinflammatory disease
- Caused by mutations in Three prime repair exonuclease 1 (Trex)
- Driven by lack of clearance of DNA from apoptotic cells by macrophages



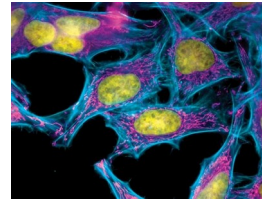
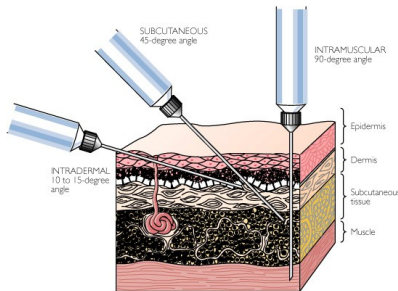
Severe neurological and developmental symptoms in AGS patients

3) DNA vaccination

Plasmid
encoding
antigen



Electroporation
Gene gun
Intramuscular
injection

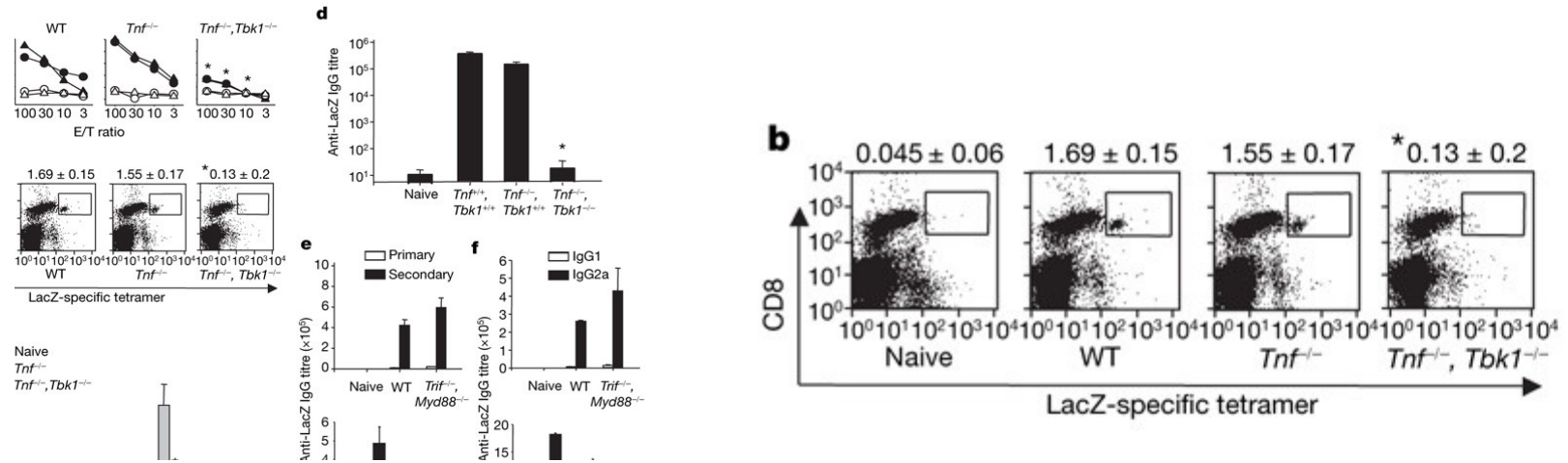


Transcription
Translation
**Antigen
presentation**

Innate immune DNA
sensing
Inflammation

**Protective immunity to
encoded antigen**

3) DNA vaccination requires cytoplasmic DNA sensing



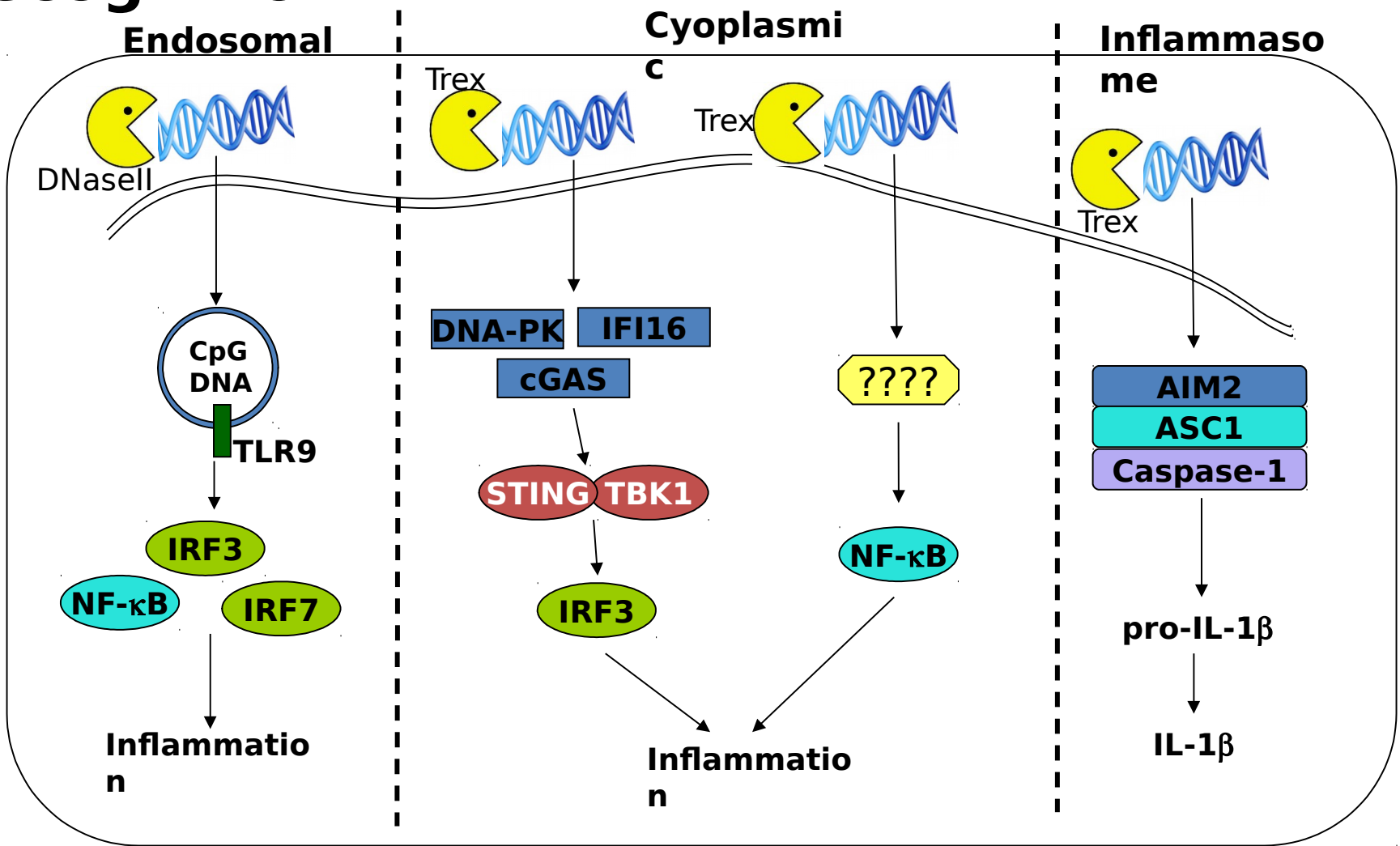
No antigen-specific CTL or IgG response to DNA vaccination in TBK1^{-/-} mouse

Similar data from STING^{-/-} and cGAS^{-/-} mice

For DNA vaccination to result in protective immunity

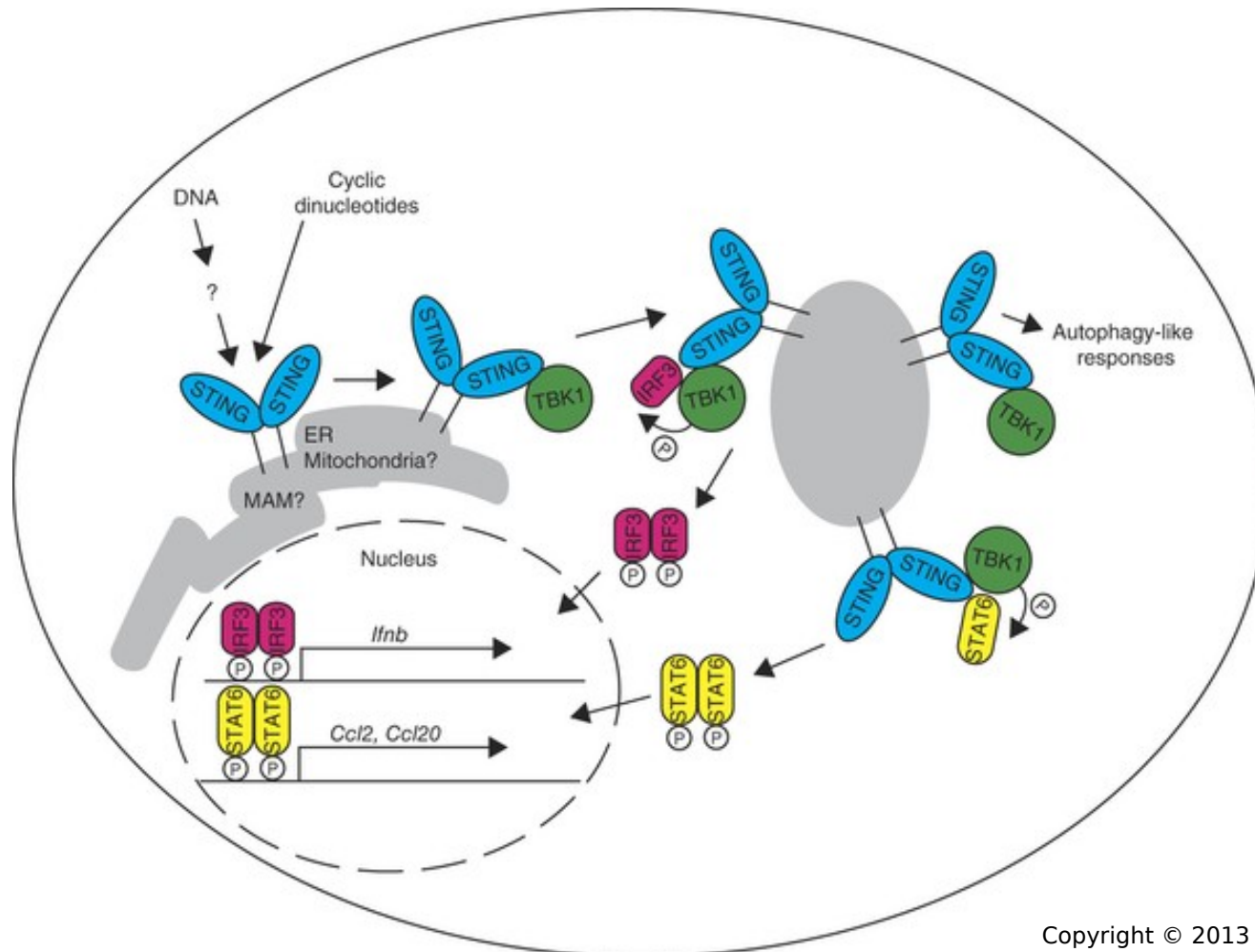
- 1) Requires entry into cells
- 2) Requires effective stimulation of intracellular DNA sensing pathways
- 3) Requires expression of encoded antigen

Molecular mechanisms of pattern recognition - DNA



Molecular mechanisms of DNA recognition

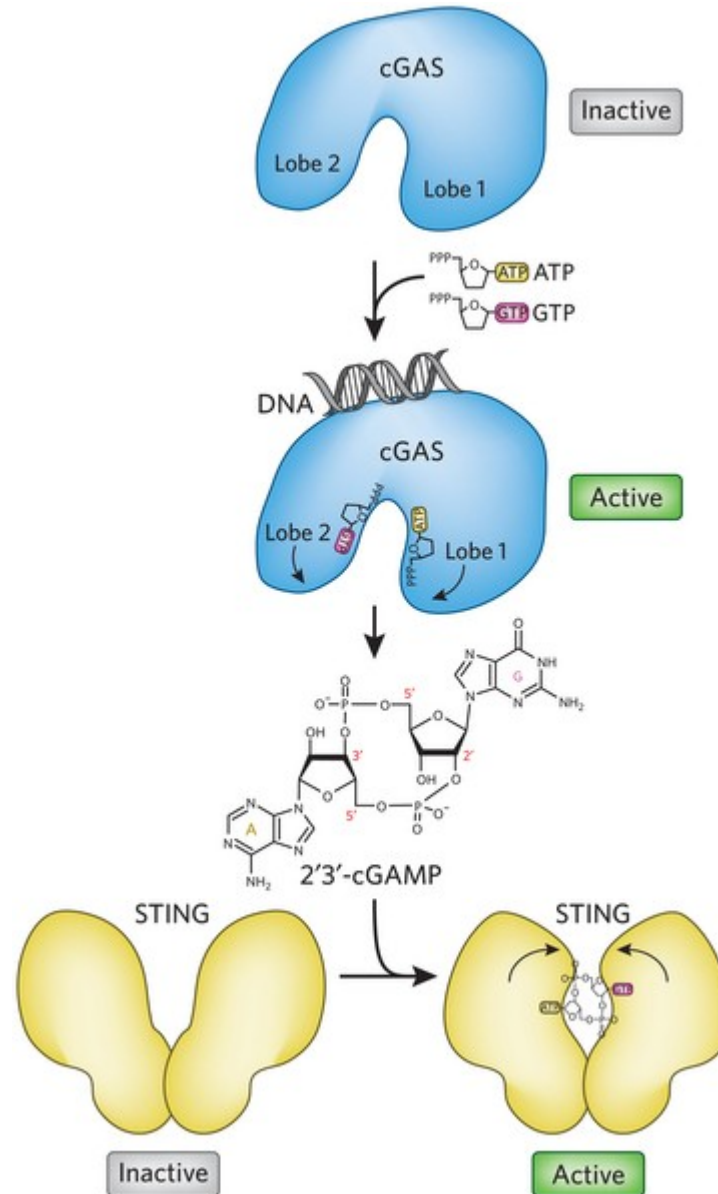
STING: Endoplasmic reticulum (and mitochondrial) resident protein – translocates to the golgi apparatus upon stimulation



Molecular mechanisms of DNA recognition - cGAS

cGAS synthesizes
cGAMP

cGAMP activates
STING



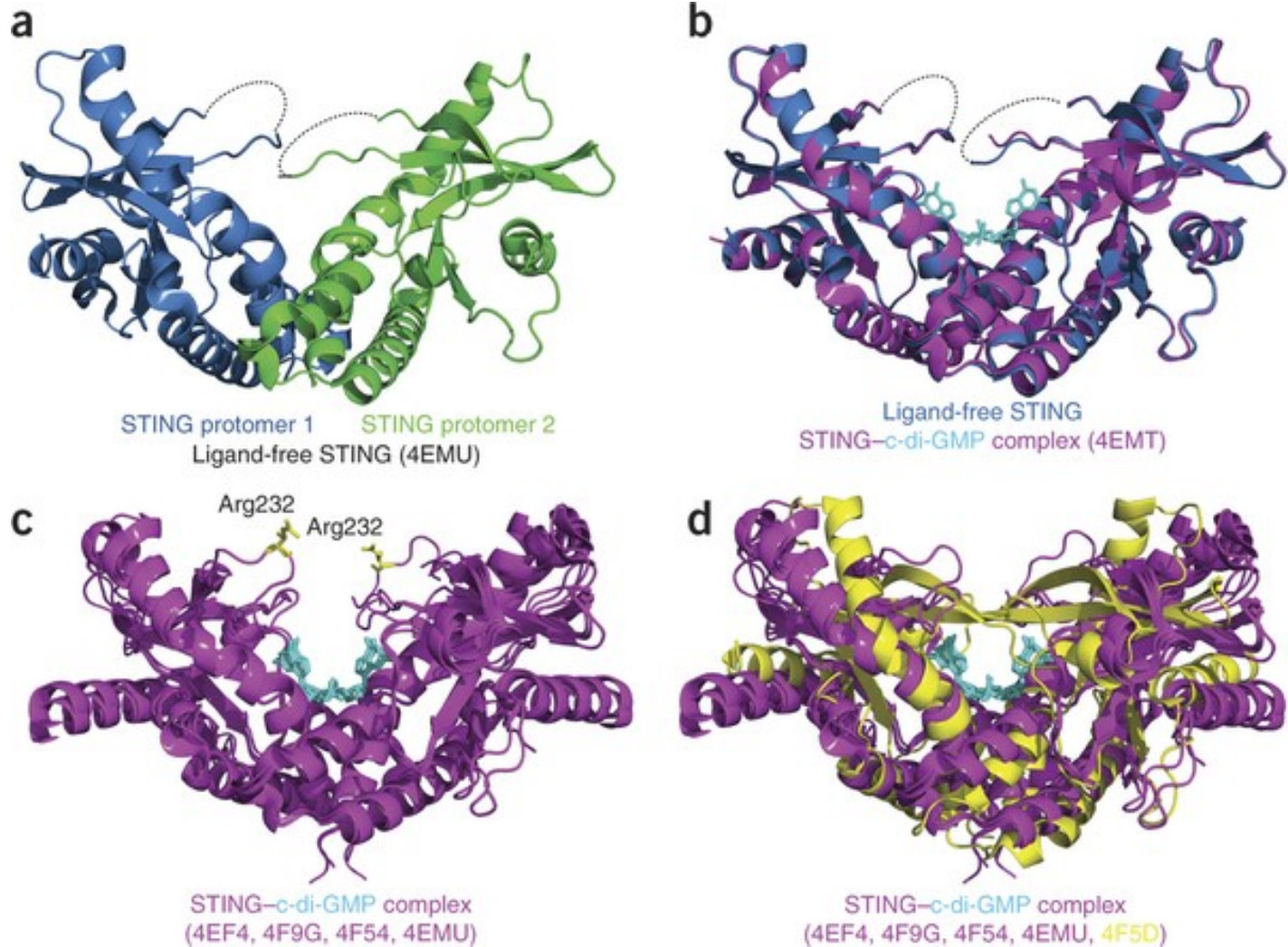
Nature Chemical biology 2013 9

533-534

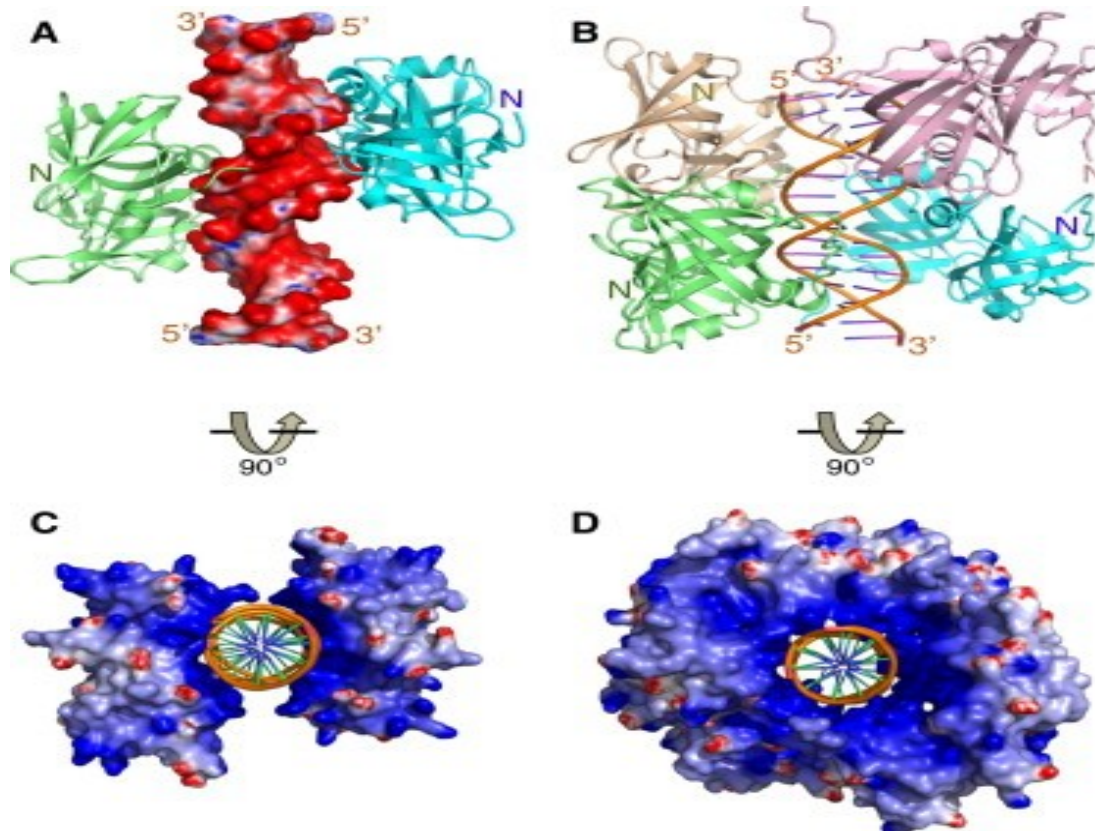
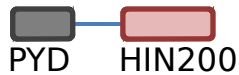
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Molecular mechanisms of DNA recognition - STING

STING structures: binds to cyclic di-nucleotides

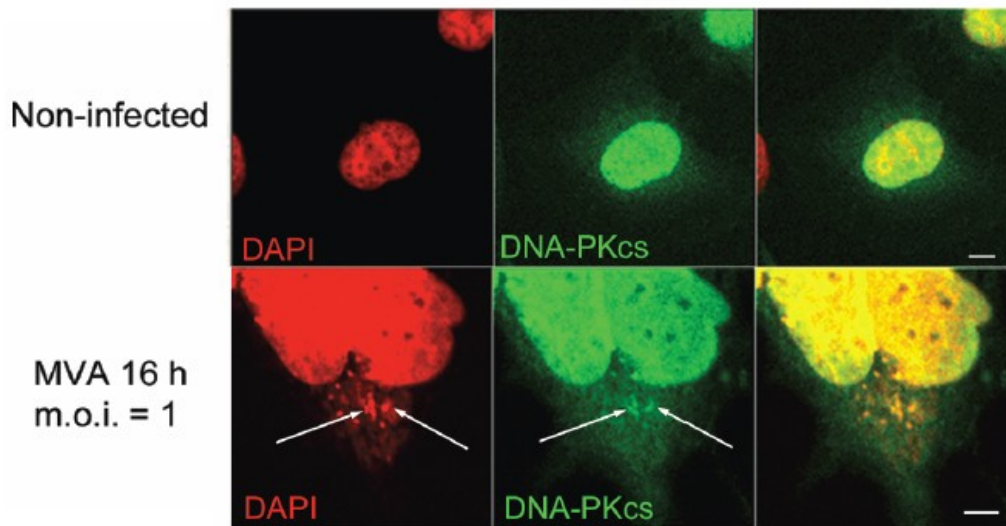
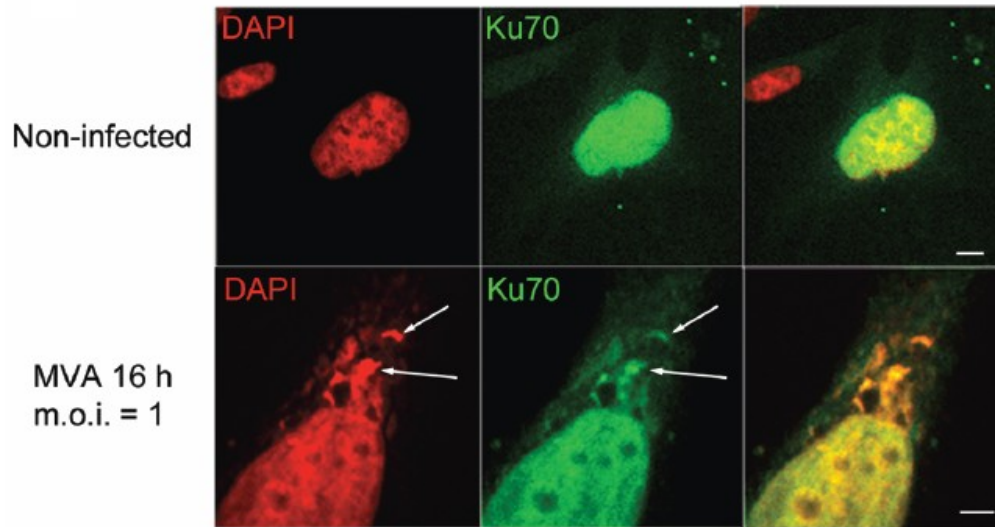


The structure of the AIM2 HIN:DNA complex (crystal form I): AIM2 binds ASC to form an inflammasome and produces IL1 plus cell death

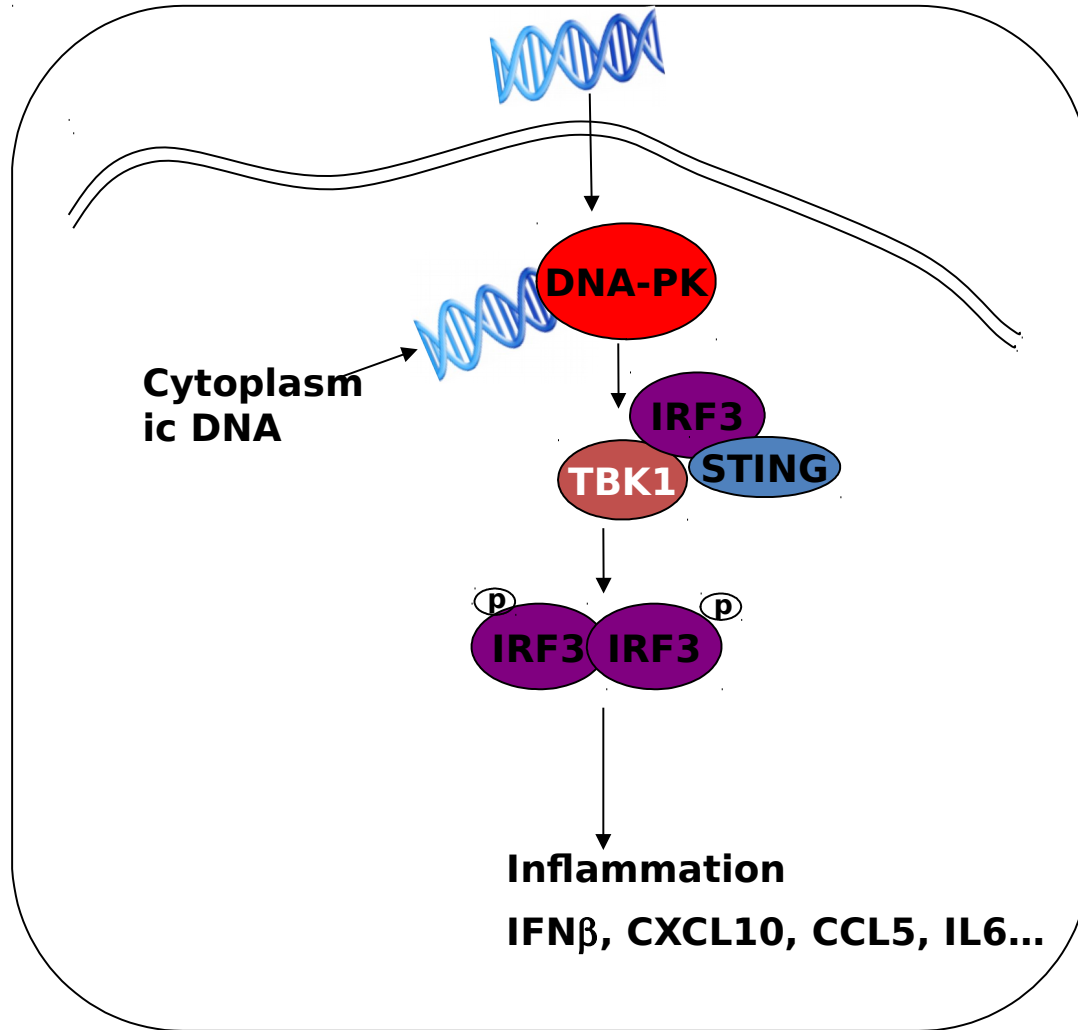


Molecular mechanisms of DNA recognition

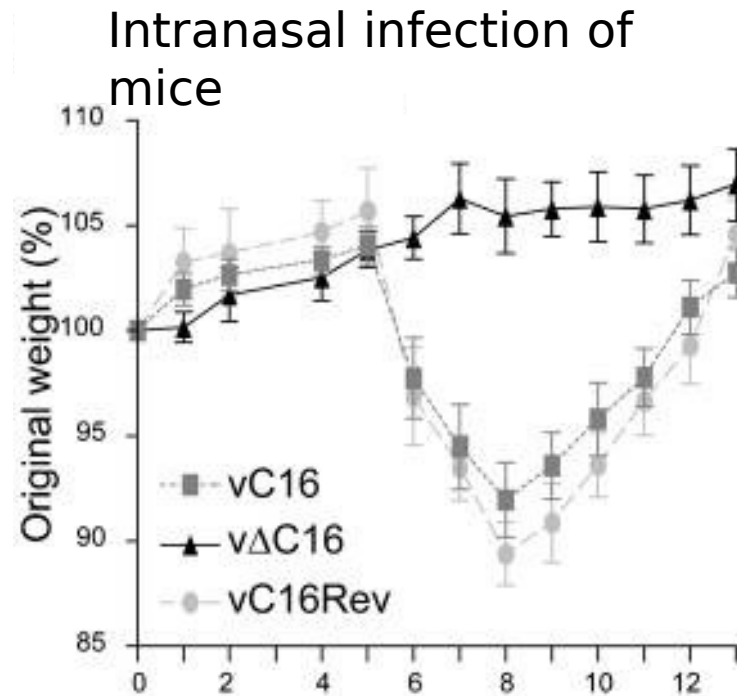
Ku and DNA-PKcs localise to viral DNA factories during MVA infection



DNA-PK / IRF3-dependent signalling

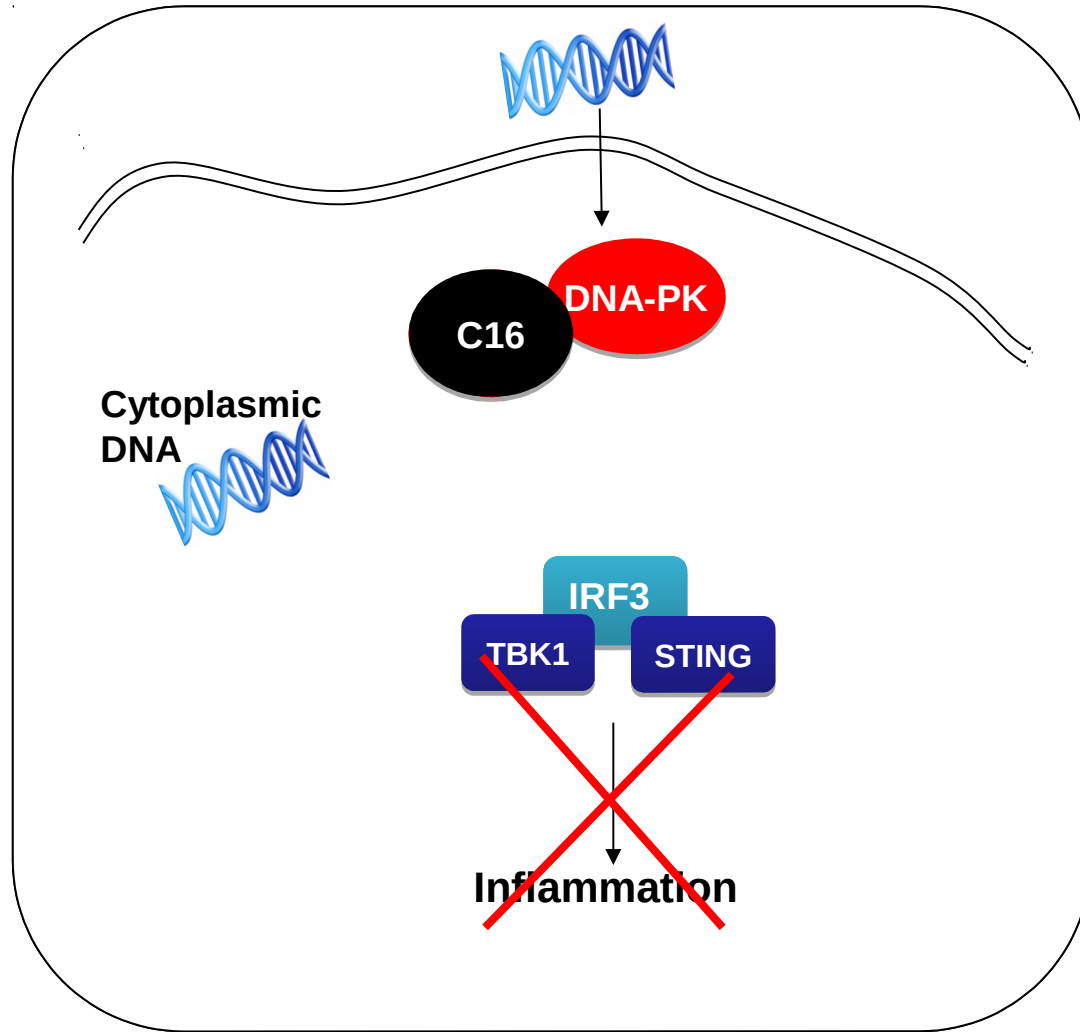


Vaccinia virus protein C16 is a virulence factor



Mice infected with VACV Δ C16 lose less weight than those infected with wt virus

C16 inhibits DNA-PK dependent DNA sensing



Take Home messages:

Nucleic acids are potently immunostimulatory – when found as non-self structures or found in the inappropriate location

Detection of DNA by innate immune system is crucial for fighting pathogens as well as for warning against tissue damage

Multiple mechanisms of detection of DNA

Cytoplasmic DNA sensing pathways are crucial for fighting infection

Dysregulation of these pathways can lead to autoimmunity, often due to a build up of DNA which triggers inflammatory signalling